Reproducibility of Retinal Nerve Fiber Layer Thickness Measurements using the Eye Tracker and Retest Function of Spectralis(R) SD-OCT in Glaucomatous Eyes and Healthy Controls

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Abstract: PURPOSE. To evaluate the impact of self-acting eyetracking and retest software on the reproducibility of retinal nerve fiber layer (RNFL) thickness measurements in glaucoma patients and healthy control subjects using Spectralis(®) SD-OCT. METHODS. RNFL thickness was measured in 56 normal and 47 glaucomatous eyes by one operator within one session with a brief rest between measurements. Three measurements were taken with eyetracker and retest function engaged (Method A), and three measurements were taken without eye tracker and without retest function (Method B). Measurements with Method A and Method B were taken alternately. RESULTS. Reliability, measured by intraclass correlation coefficient (ICC) for absolute agreement and coefficient of variation (COV), was calculated for the globalmean RNFL thickness (G), for each sector and for the peripapillaryboundle. The ICC (and lower 95% confidence interval (CI)) for the global mean RNFL thickness(G) for measurements using Method A in both normal and glaucomatous eyes was 0.99 (0.98 CI). In glaucomatous eyes the COV for measurements using Method B was between 2.7% and 10.5%, and between 1.3% and 3.5% formeasurements usingMethod A. CONCLUSION. Reproducibility of RNFL measurements with Spectralis(®) SD-OCT is excellent in bothnormal and glaucomatous eyes and can be significantly improved by using its eye trackerand retest software. The gain of reproducibility by using the software is significantly higher in glaucomatous eyes than in normal eyes. These findings suggest that software applications are capable ofsignificantly improving reproducibility of RNFL thickness measurements.

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Reproducibility of Retinal Nerve Fiber Layer Thickness Measurements using the Eye Tracker and Retest Function of Spectralis® SD-OCT in Glaucomatous Eyes and Healthy Controls.

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ABSTRACT

PURPOSE. To evaluate the impact of self-acting eyetracking and retest software on the reproducibility of retinal nerve fiber layer (RNFL) thickness measurements in glaucoma patients and healthy control subjects using Spectralis® SD-OCT.

METHODS.RNFL thickness was measured in 56 normal and 47 glaucomatous eyes by one operator within one session with a brief rest between measurements. Three measurements were taken with eyetracker and retest function engaged (Method A), and three measurements were taken without eye tracker and without retest function (Method B). Measurements with Method A and Method B were taken alternately.

RESULTS. Reliability, measured by intraclass correlation coefficient (ICC) for absolute agreement and coefficient of variation (COV), was calculated for the globalmean RNFL thickness (G), for each sector and for the peripapillaryboundle. The ICC (and lower 95% confidence interval (CI)) for the global mean RNFL thickness(G) for measurements using Method A in both normal and glaucomatous eyes was 0.99 (0.98 CI). In glaucomatous eyes the COV for measurements using Method B was between 2.7% and 10.5%, and between 1.3% and 3.5% formeasurements usingMethod A.

CONCLUSION. Reproducibility of RNFL measurements with Spectralis® SD-OCT is excellent in bothnormal and glaucomatous eyes and can be significantly improved by using its eye trackerand retest software. The gain of reproducibility by using the software is significantly higher in glaucomatous eyes than in normal eyes. These findings suggest that software applications are capable ofsignificantly improving reproducibility of RNFL thickness measurements.

INTRODUCTION

Glaucoma is a widespread ophthalmicdisease leading to progressive loss of visual field function. The deathof retinal ganglion cells culminates in the loss of visual acuity making glaucoma one of the main causes of irreversible blindness in industrialized nations and worldwide.[1, 2] Elevated intraocular pressure (IOP) is a major risk factor for the onset of glaucoma.[3, 4] In addition to IOP, other risk factors are well known, such as age, family history and race.[5] Primary open angle glaucoma (POAG) and Primary angle closure glaucoma (PACG) are the main forms of glaucoma in the world. Pseudoexfoliationglaucoma(PEX) is the main cause of secondary open angle glaucoma.

By the time the lossof retinal ganglion cells is clinically detected, extended and irreversible damage has already occurred.[6, 7]Since effective therapy can protract the progress of glaucoma, early diagnosis is one of the main goals in the treatment of this disease. It is strongly believed that the thinning of the retinal nerve fiber layer (RNFL) correlates highly to,or even precedes visual field loss in glaucoma.[8-14] Therefore, establishing reliable methods of RNFL measurement could be one key step in early diagnosis and treatment of glaucoma.

Using optical coherence tomography (OCT), Huang et al. were the first to present a non-contact, non-invasive method of using low-coherence interferometry to determine the echo time delay and magnitude of backscattered light reflected off different layers of a structured tissue sample.[15] The unique optic free pathway through the eye made OCT highly applicable to the visualization of retina layers. In 1995, time domain OCT (TD-OCT) was introduced as an imaging technique for glaucoma diagnosis.[13] In spectral domain OCT (SD-OCT or Fourier domain OCT), a

moving reference mirror as used in TD-OCT is no longer needed.[16, 17] SD-OCT provides higher resolution at faster scanning speeds.[18]

Another invention available in recent OCT devices is the implementation of specific algorithms and software to further enhance scanning resolution and decrease motion artifacts. In 2006, Spectralis® SD-OCT (Heidelberg Engineering GmbH, Heidelberg, Germany) was introduced for retinal imaging. This instrument features two different options to enhance reproducibility. An online eye tracking device (eye tracker) compensates for involuntary eye movements during the scanning process, and a retest function assures that follow-up measurements are taken from the same area of the retina as the baseline examination. Both options can be switched off. The aim of this study was to test the impact of using both the eye tracker and retest functions on the reproducibility of RNFL thickness measurements performed with Spectralis® SD-OCT.

METHODS

Subjects

The study was conducted in accordance withthe guidelines of the Declaration of Helsinki and was approved by the local ethics committee. Written informed consent was obtained from each subject. Subjects were recruited from the ophthalmological out-patient service of the UniversityHospital Zurich, Switzerland. All subjects underwent a full ophthalmological examination including measurement of refraction, best corrected visual acuity, intraocular pressure (Goldman applanation tonometry) and a slit lamp examination of the anterior and posterior segment.

The inclusion criteria for both healthy and glaucomatous subjects were a visual acuity of more or equal to 0.5 Snellen, refractive error less than ±5.00 diopters (D) spheric and ±3.00 D cylindrical and no history of ocular trauma or of any other severe ocular disease (particularly diseases affecting the optic nerve or surgery other than uncomplicated cataract surgery). Additional inclusion criteria for the glaucoma group were a diagnosis of primary open angle glaucoma (POAG) or PEX-glaucoma. All glaucoma patients underwent visual field (VF) testing. Young patients in the control group with normal optic disc and no or nearly no cupping were included into the studywithout VF testing. If IOP or the cupping was abnormal or even if there was a difference in sides, VF was tested in control patients.Regarding the VF tested with G1-program by Octopus perimeter (Haag-Streit AG, Koeniz, Switzerland),the mean defect (MD) for control patientshad to be less than or equal to 2.0dB and greater than 2.0dB for glaucoma patients. Exclusion criteria for the control group were history of glaucoma or intraocular pressure beyond 21 mmHg and optic disc cupping of more than 0.6. Age was not an exclusion criterion.

In general,glaucoma was defined based on a clinical diagnosis of a progressive optic nerve damage with abnormal optic disc cupping and MD >2.0dB. We assessed the optic disc in accordance withthe paper of Jonas et al.[19]Primary open-angle glaucoma is characterized as anoptic nerve neuropathy with atrophy of the optic nerve and loss of retinal ganglion cells and axons and in combination with characteristic visual field abnormalities. The anterior chamber angle is open.[20]PEX-glaucoma is defined as elevated IOP causing progressive optic nerve damage. In PEX-glaucoma, IOP elevation is caused by abnormal protein obstructing the trabecular meshwork.

Image and Data Acquisition

All RNFL circle scans were performed by one examiner using the Spectralis® SD-OCT system (Heidelberg Engineering GmbH, Heidelberg, Germany, software version 5.1.2.). A pupil diameter of at least 4 mm was required for scanning. Within one session, three measurementswere taken with eye tracker and retest function engaged (Method A) and three measurements were taken without eye tracker and without retest function with manual positioning and repositioning of the scanning circle (Method B). Measurements performed with Method A were alternated by measurements performed with Method B to avoid systematic bias.

SD-OCT RNFL thickness measurements were performed by using circlular scans with a scanning angle of 12° which equates a retinal diameter of 3.5 mm when assuming a standard corneal curvature of 7.7mm.Using a super luminescent diode with a center wavelength of 840 mm, the Spectralis® SD-OCT obtains up to 40,000 A-scans/sec with a depth resolution of 7µm in tissue and a transversal optical resolution of 14µm in an average human eye. The transversal digital resolution depends on the tightness of calculated A-scans (pixels) and can be adjusted. In high resolutionmode (HR-mode) the device provides a transversal digital resolution of 5µm. High speed mode (HS-mode) doubles the distance between A-scans. As a result, transversal digital resolution decreases to 11µm. A full RNFL circle scan contains 1536 A-scans in HR-mode and 768 A-scans in HS-mode along a peripapillary circle of 360°. In the present study, all scans were performed in HS-mode, as HS-mode is commonly used for RNFL examinations in the utilized clinic.

With dual-beam, a corresponding scanning laser ophthalmoscope (SLO) fundus image can be captured at the same time as the OCT measurement, enabling the

system to link every OCT scan (Fig 1A) to its corresponding position on the SLO fundus image (Fig 1B). Processing of SLO data and identification of specific patterns in retinal structures such as blood vessels allows scans to be marked as a reference and baseline. In follow-up examinations, the system recognizes the former scanning area on the retina and automatically positions the retest scan on the same location. During the measurement, online eye tracking provides a real-time adjustment of the OCT scanner on the simultaneously gathered SLO image in order to decrease motion artifacts, while the high scanning speed reduces the time for distracting eye movements to occur. To increase image quality, the Spectralis® SD-OCT includes an ART function (automatic real time). With ART activated, multiple frames (B-scans) are gathered during the scanning process and images are averaged for noise reduction.[21, 22]The number of frames can be adjusted. In this study, the ART function was set to 16 frames per B-scan for all measurements carried out.

The first A-scan is processed temporally (0°), after which the scanner moves through superiorly (90°), nasally (180°), inferiorly (270°) and back to temporally to complete a full circle. During the measurement, a quality bar visualizes the signal to noise ratio. The quality scores range from 0 (poor quality) to 40 (excellent quality). For this study, scans with a quality of less than 25 were excluded and repeated until good quality was achieved. Likewise, scans with blinks during the scanning process were excluded and repeated.

For the first RNFL measurement, the OCT scanning circle was manually positioned at the center of the optic disc while eye tracking was activated. The first acquired RNFL scan was set as a baseline for further retest scans (Method A). Every retest was performed alternately with a “traditional” scan with manual repositioning of the

scanning circle on the optic disc (Method B). Between each measurement, the subject was instructed to lean back before being repositioned on the headrest and the correction for spherical errors was readjusted.

The Spectralis® SD-OCT system provides an algorithm to determine the inner and outer boundaryof the RNFL (Fig 1A). OCT data was analyzed by this algorithm to detect RNFL thickness along the circular scan in micrometers. The median thickness was plotted in a pie chart diagram representing the six sectors of the optic disc (Fig 1D). For interpretation of the RNFL scan the optic disc is segmented as follows: temporal (T, 315° to 45°), superior temporal (TS, 45° to 90°), superior nasal (NS, 90° to 135°), nasal (N, 135° to 225°), inferior nasal (NI, 225° to 270°), inferior temporal (TI, 270° to 315°), together with an averaged global classification (G) and the papillomacular bundle (PMB, 338° to 8°). The calculated thickness profile (Fig 1C) refers to a set of normative RNFL thickness data collected from measurements in 170 healthy subjects.[23] Green areas represent the 95% normal range found in healthy subjects of the same age whereas values outside the 99% confidence interval of the normal distribution ($0.01 < p < 0.05$) are indicated in red. Yellow areas represent values outside the 95% confidence interval but within the 99% confidence interval of the normal distribution.

Statistical Analyses

For statistical analyses, the RNFL thickness of all sectors, the papillomacular bundle (PMB) and the global mean RNFL thickness (G) were analyzed. Analyses were conducted using PAWS/SPSS Statistics version 18 (IBM Corporation, New York, USA) and MedCalc version 11.2 (MedCalc Software, Mariakerke, Belgium).

A pilot study was conducted (5 eyes of 5 healthy controls and 5 eyes of 5 patients with a glaucoma diagnosis) to calculate the sample size that would be needed to distinguish a relevant difference in COV of ±1% between Method A and Method B. Sample size calculation was based on the measurements of measurement area G(global meanRNFL-thickness) in glaucomatous eyes according to the same examination protocol as described in the main study. The pilot study showed that when the sample size in the glaucoma group is 28, a single group t-test with a 0.05 two sided significance level will have 95% power to detect the difference between a null hypothesis mean of 2.000and an alternative mean of 1.000, assuming that the standard deviation is 1.400.

Descriptive statistics for quantitative variables such as means and standard deviations as well as relative frequencies for qualitative variables such as sex were conducted. A Student's t-test was calculated for differences in age between healthy and glaucomatous eyes.

One set of three RNFL thickness values obtained from three measurements using the same measurement method (Method A or Method B) in the same eye was used to calculate an intraclass correlation coefficient (ICC) and the coefficient of variation (COV). COV was defined as standard deviation divided by the arithmetic mean and was expressed in percentage. For both the glaucoma group and the control group this resulted in a population of COV values for measurements acquired by Method A and another population of COV values for measurements acquired by Method B. Thus the population statistics for COV were calculated. Such calculations were done separately in all measurement areas (G, TS, T, TI, NI, N, NS and PMB).

Consequently, differences in the mean COV between the two measurement methods (Method A minus Method B) were computed.

Moreover, differences between the mean RNFL thickness in µm provided by Method A and Method B were computed for all measurement areas in both glaucoma group and healthy controls.

A one sample Student's t-test was applied to the differences of means to determine if Method A provides different measurement than Method B. Moreover, agreement of Method A and Method B was investigated with a Bland & Altman plot along with the corresponding 95% limits of agreement.[24] A one sample Student's t-test was also applied to COV differences in order to determine if Method A was more reproduciblethan Method B for healthy subjects and glaucoma patients (corresponding to a paired t-test). A two sample t-test was used to explore possible differences in COV reduction (Method A, Method B) between the healthy controls and the glaucoma group. A two sample t-test was used to determine if significant differences existed in thickness (Method A versus Method B) between healthy and glaucomatous eyes.

A linear regression analysis for reproducibility(COVs) and global mean thickness (G) with age and sex as predictors was conducted in order to find associations between predictors and COVs for every sector, the PMB and for global mean RNFL thickness (G) for Method A separately for healthy and glaucomatous eyes.

A one-way ANOVA with aScheffè post-hoc test was conducted to investigate potential differences in the reproducibilityof sectors for both eye tracker and traditional measurement.

Results of the statistical analyses with p-values under 0.05 were interpreted as statistically significant.

RESULTS

Demographic parameters

One hundred and seven subjects were examined. Four patients had to be excluded, three due to poor fixation and one due to poor measurement quality and failure of RNFL thickness algorithm. Forty-two left and 61 right eyes were included. Table 1 displays the characteristics and demographics of the study population.

A Student's t-test showed a significant difference in age between glaucoma patients and healthy controls ($p = 0.007$); whereas there was no significant difference in age between men and women ($p = 0.813$) in either group.

Reliability of measurements performed with eyetracker and retest function

The mean RNFL thickness is shown in Table 2 for each sector, the PMB and global mean thickness (G). As Table 2 shows, RNFL thickness measured higher in superior and inferior sectors compared to temporal and nasal sectors in both groups, regardless of the measurement method. Within both the glaucoma and the control groups, RNFL thickness values were very similar using each method (Table 2). RNFL thickness values in the glaucoma group were significantly decreased in all quadrants compared to healthy controls (mean RNFL decrease of 24µm, $p < 0.001$).

Figure 2 shows a Bland & Altman plot for differences in mean RNFL thickness in µmfor both methods alongwith the corresponding 95% limits of agreement. Neither method was significantly biased towards thicker or thinner RNFL thickness values.

Reproducibility of RNFL measurements

Table 3 shows the COVs calculated from the three measurements. In healthy subjects, COVs for RNFL thickness measurement of the six sectors of the optic disc (T, TS, TI, N, NS, NI) measured withMethod B (without eye tracker and without retest function, with manual positioning of the scanning circle) ranged from 3.5% (temporal inferior sector, SD ± 0.023) to 7.4% (nasal, SD ± 0.051). COVs from measurements with Method A (with eye tracker and retest function engaged) ranged from 1.0% (temporal superior, SD ± 0.012) to 2.5% (nasal, SD ± 0.017). In glaucoma patients, COVs for measurements with Method B ranged from 5.8% (temporal, SD ± 0.034) to 10.5% (nasal superior, SD ± 0.110); whereas COVs for measurements with Method A ranged from 1.6% (temporal, SD± 0.015) to 3.8% (temporal inferior, SD ± 0.040). For measurements in both groups with both methods, the COVs calculated for the global mean RNFL thickness(G) were even lower than in separate calculations for the sectors (1.6%, respectively 1.0% in healthy subjects; 2.7%, respectively 1.3% in glaucoma patients).

Effect of eye tracker and retest function on reproducibility and RNFL thickness measured

Differences in mean RNFL thickness and COVs between the two measurement methods are presented in Table 4. Significant differences in mean RNFL thickness between the two measurement methods were found in the control group for global

mean RNFL thickness (G, Method A +0.565 µm, p = 0.043) and sectors TS (-2.042 µm, p = 0.018), NI (+3.464 µm, p = 0.002) and N (+2.304 µm, p = 0.009). There was no significant difference in mean RNFL thickness between the two measurement methods withinthe glaucoma group. Differences of COVs for the two measurement methods (Method A minus Method B) were all negative, providing strong evidence that Method A is more reproduciblethan Method B Figure 3 displays the reduction of COVs by the Method A in a bar plot, together with its corresponding 95% CI.

Linear regression analysis did not unveil any significant effect of sex nor age on reproducibility(COV) of measurements with Method A or Method B.

For Method A, ANOVAs revealed that there are no differences in COV between sectors. For Method B there are differences (p = 0.001) in COVbetween sectors for both healthy subjects and glaucomatous patients. The TI sector (temporal inferior) is the highest reproducible sector measured.

DISCUSSION

Since its introduction in 1991, OCT technology has experienced a dramatic evolution and has quickly becomea popular resource in ophthalmologic imaging and diagnostics. In spite of its popularity and OCT's role in glaucoma research, its role in diagnostics is still developing. The correlation between glaucoma progression and RNFL thinning is well-documented.[8, 11] Evidence that the thinning of RNFL precedes loss of visual field function indicated that OCT could help detectthe onset of glaucomatous changes more sensitively than by testing the visual field.[12] An earlier diagnosis of glaucomatous changes gives clinicians more time to establish an effective therapy and may help maintain patients'visual field function.

Previous studies have kept record of the increasing reproducibility of RNFL thickness measurements performed with first-[25, 26], second-[27, 28] and third-generation[29] TD-OCT technology as well as for the different SD-OCT devices[30-35] that are currently available. The aim of this study was to investigate the contribution of twospecific software applications to achieve more reliable and higher reproducible RNFL thickness measurements. The improvements of SD-OCT have been well documented in the literature.[30-35] While other studies have already been published on the reproducibility of RNFL thickness measurements with SD-OCT, the uniqueness of the present study lies in the differentiation between the two measurement modalities (Method A and Method B). Higher resolutions and faster scanning speeds of SD-OCT have made measurements more reproducible. The present study demonstrates that reproducibility can be further improved by the use of specific software for retest recognition and compensation for involuntary eye movement (eye tracker).

The Spectralis® Software algorithm automatically detects RNFL. In some cases the software hasproblems detecting the corrected boundaryof the RNFL. In these cases, it is possible to manually correct the boundaryin the Spectalis® software. To avoid bias by a glaucoma specialist using manual correction of the RNFL boundary, we did not do this in the presented study. If it wasobvious that the automatic detectionfailed,thosestudy eyes wereexcluded.

Our results show good reliability for measurements usingeye tracker and retest function (Method A). The Bland and Altman plot with 95% limits of agreement (Figure 2) shows that RNFL-thickness values for the global measurement area (G) are

comparable between the two methods (Method A and Method B). The only significant difference in RNFL thickness was found in control patients where Method A resulted in higher values for the inferior nasal sector (NI), nasal sector (N) and mean global RNFL thickness (G), and lower values in the temporal superior sector (TS). As the differences are as low as 0.2 to 3.0µm, we consider them to be clinically irrelevant. Measurements with the new eye tracker and retest functions(Method A) are reliable and comparable to the thickness values measured without eye tracker and without retest function(Method B).

Results of this study show excellent reproducibility and significant improvement of reproducibility of RNFL measurements by using the eye tracker and retest software. Using eye tracker and retest software (Method A) of Spectralis® SD-OCT, enhances reproducibility significantly (COV 2.7% to 1.3% for global mean RNFLthickness (G) in glaucomatous eyes, p=0.000). In glaucoma patients, the improvement of reproducibility was significantly higher than in controls. To our knowledge the present study is the first to report on the reproducibility of RNFL measurements using the Spectralis® SD-OCT device.

Budenzet al.[29] studied reproducibility of RFNL measurements with TD-OCT Stratus OCT3 (Carl Zeiss Meditec, Dublin, California) in 88 normal and 59 glaucomatous eyes and found COVs ranging from 3.7% (global mean RNFL thickness G) to 11.9% (nasal quadrant N) in glaucomatous eyes and from 1.7% (global mean RNFL thickness G) to 8.25% in normal eyes, respectively. Similar to the present study, the nasal quadrant was the least reproducible, whereas the temporal quadrant was the most reproducible. Also in that study, glaucomatous eyes showed less reproducibility than normal eyes, which was also reported by Blumentalet al.[27] Though

reproducibility found in the present study is still higherin normal eyes than in glaucomatous eyes, our results show that eye tracker and retest software (Method A) had a higher impact on reproducibility in glaucomatous eyes than in normal eyes. This indicatesthat such software may help to reduce the gap in reproducibility previously found between measurements of glaucomatous and normal eyes. Mwanza and collegues[35] studied intravisit and intervisit reproducibility of RNFL thickness and optic nerve head parameters measured with the SD-OCT Cirrus HD-OCT (Carl Zeiss Meditec, Inc., Dublin, California) in 55 glaucomatous eyes. Similar to the present study, the mean RNFL thickness showed best intravisit reproducibility with a COV of 1.9%. Menkeet al. reported on the reproducibility of the 3D Fourier-Domain OCT (3D OCT1000; Topcon, Tokyo, Japan) in 38 normal subjects performing 3 RNFL thickness measurements by two different operators.[34] The mean COV was 4.1%. Highest reproducibility was found for the inner ring area (ETDRS-scheme) with a COV of 1.9%, which compares with results of the present study for measurement Method B (without eye tracker and without retest function) in normal eyes (1.6%). Lee et al. used the test-retest function of Spectral OCT/SLO (Ophthalmic Technologies Inc, Toronto, Canada) to investigate reproducibility of RNFL thickness measurements in 98 normal and 79 glaucomatous eyes performing three measurements within one session.[32] RNFL measurements showed good reproducibility for that device. As in the present study, best reproducibility was found for the global mean RNFL thickness, with a COV of 1.9% in normal and 2.0% in glaucomatous eyes. As all scans were performed with one method (retest) only, no conclusion regarding the specific effect of the retest function to enhance reproducibility could be drawn.

Reproducibilities found in different studies are not directly comparable, as different eyes and different study protocols are used. The results of the present study showed excellent reproducibility for measurements using the eye tracker and retest protocol (Method A) of Spectralis® SD-OCT. With lowest COVs of 1.0% in normal eyes and 1.3% in glaucomatous eyes, the results show one of the best reproducibilities ever reported for RNFL thickness measurements in any OCT device available today.HS-mode used in the present study only utilizes half the transversal resolution the device is capable of. If one were to use the full resolution of HR-mode, even higher reproducibility might be achieved. While other currently available OCT devices provide a scanning resolution comparable to the device used in the present study and also include test-retest software, the Spectralis® SD-OCT is the first device to integrate real-time eye tracking. The significant improvement of reproducibility attained by using this software in the present study indicates that improvement in reproducibility cannot entirely be accounted for by higher resolutions and faster scanning speeds of the latest SD-OCT devices, but also has to be understood as a result of more sophisticated software applications. These findings may have implications for the design and development of the next generation of OCT devices.

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Table 1. Demographic characteristics of glaucoma patients and healthy subjects

	Control	Glaucoma	Total subjects
Eyes (n)	56 (54.37%)	47 (45.63%)	103
Mean age (y)*	46,31 ± 19,56	68,96 ± 16,22	56,65 ± 21.30
Female (n)	28 (50%)	23 (49%)	51 (49.5%)

*± Standard derivation (SD)

Table 2. Mean RNFL-thickness in micrometers as the mean ± SD for both examination methods in glaucoma patients and controls

	Controls		Glaucoma	
	Method B*	**Method A†**	**Method B***	**Method A†**
G‡	95 ± 11.6	96 ± 12.6	71 ± 16.5	71 ± 16.9
TS	130 ± 17.8	128 ± 18.2	96 ± 29.4	96 ± 28.7
T	71 ± 10.8	70 ± 11.2	61 ± 14.6	60 ± 183.9
TI	138 ± 19.2	138 ± 20.2	91 ± 36.7	90 ± 37.1
NI	103 ± 23.7	106 ± 26.2	75 ± 20.1	77 ± 23.0
N	71 ± 15.8	73 ± 18.2	56 ± 17.0	57 ± 18.9
NS	108 ± 25.8	108 ± 28.4	70 ± 26.4	72 ± 28.3
PMB	55 ± 9.9	54 ± 11.3	51 ± 11.2	50 ± 11.7

*Method B = Measurements without eye tracker and without retest software, with manual positioning of the scanning circle
†Method A = Measurements with eye tracker and retest software engaged

‡G = 0-360°, T = 315°-45°, TS = 45°-90°, NS = 90°-135°, N = 135°-225°, NI = 225°-270°, TI = 270°-315°, PMB = 338°-8°

Table 3. Intraclass Correlation Coefficient (ICC) and Coefficient of variation (COV) for measurements using eye tracker and retest software and measurements without eye tracker and without retest software in glaucoma patients and controls

	Controls				Glaucoma			
	Method B*		Method A†		Method B*		Method A†	
	ICC§	COV	ICC§	COV	ICC§	COV	ICC§	COV
G‡	0.98 (0.96)	1.6%	0.99 (0.98)	1.0%	0.98 (0.96)	2.7%	0.99 (0.98)	1.3%
TS	0.86 (0.79)	4.4%	0.99 (0.79)	1.4%	0.92 (0.88)	6.5%	0.99 (00.99)	1.8%
T	0.83 (0.75)	5.6%	0.99 (0.97)	1.5%	0.92 (0.87)	5.8%	0.99 (0.98)	1.6%
TI	0.91 (0.87)	3.5%	0.97 (0.95)	1.4%	0.97 (0.97)	5.9%	0.98 (0.96)	3.5%
NI	0.91 (0.86)	6.3%	0.98 (0.97)	2.1%	0.88 (0.87)	8.7%	0.99 (0.98)	2.3%
N	0.83 (0.75)	7.4%	0.99 (0.97)	2.5%	0.89 (0.83)	9.5%	0.99 (0.98)	3.0%
NS	0.91 (0.86)	6.0%	0.99 (0.98)	2.1%	0.89 (0.83)	10.5%	0.99 (0.98)	3.8%
PMB	0.85 (0.77)	6.5%	0.93 (0.89)	3.0%	0.84 (0.75)	7.9%	0.97 (0.95)	2.8%

*Method B = Measurements without eye tracker and without retest software, with manual positioning of the scanning circle
†Method A = Measurements with eye tracker and retest software engaged

‡G = 0-360°, T = 315°-45°, TS = 45°-90°, NS = 90°-135°, N = 135°-225°, NI = 225°-270°, TI = 270°-315°, PMB = 338°-8°
§ICC, with lower 95% confidence intervalin parentheses;

Table 4. Differences in COV (Method A[†] – Method B[*]) and mean RNFL thickness for glaucoma patients and healthy controls

		Healthy controls				Glaucoma patients			
		One sample Student's t-test on differences				One sample Student's t-test on differences			
		p-value	Mean difference	95% CI lower	95% CI upper	p-value	Mean difference	95% CI lower	95% CI upper
Differences of meanCOV	G[‡]	< 0.001	-0.005	-0.007	-0.003	< 0.001	-0.015	-0.021	-0.008
	TS	< 0.001	-0.030	-0.038	-0.023	< 0.001	-0.047	-0.064	-0.030
	T	< 0.001	-0.041	-0.05	-0.033	< 0.001	-0.042	-0.052	-0.032
	TI	< 0.001	-0.021	-0.029	-0.013	0.210	-0.024	-0.061	0.014
	NI	< 0.001	-0.042	-0.052	-0.032	< 0.001	-0.064	-0.082	-0.046
	N	< 0.001	-0.049	-0.063	-0.035	< 0.001	-0.065	-0.084	-0.046
	NS	< 0.001	-0.040	-0.049	-0.031	< 0.001	-0.068	-0.100	-0.034
	PMB	< 0.001	-0.036	-0.049	-0.022	< 0.001	-0.051	-0.066	-0.037
Differences of mean RNFLthickness	G	0.043	0.565	0.017	1.114	0.357	0.443	-0.515	1.403
	TS	0.018	-2.042	-3.726	-0.358	0.941	0.082	-2.138	2.302
	T	0.218	-0.667	-1.739	0.407	0.196	-1.078	-2.731	0.576
	TI	0.601	0.417	-1.171	2.005	0.267	-1.163	-3.246	0.921
	NI	0.002	3.464	1.367	5.562	0.136	2.082	-0.680	4.844
	N	0.009	2.304	0.607	4.000	0.237	1.177	-0.800	3.155
	S	0.635	-0.500	-2.597	1.594	0.112	1.869	-0.454	4.193
	PMB	0.517	-0.315	-1.284	0.654	0.292	-1.138	-3.286	1.011

[*]Method B = Measurements without eye tracker and without retest software, with manual positioning of the scanning circle
[†] Method A = Measurements with eye tracker and retest software engaged

‡G = 0-360°, T = 315°-45°, TS = 45°-90°, NS = 90°-135°, N = 135°-225°, NI = 225°-270°, TI = 270°-315°, PMB = 338°-8°

Figure 1. Circular peripapillary optical coherence tomogram of a normal right eye (1A) with its corresponding fundus image (1B) obtained with the dual beam scanning laser ophthalmoscope. Red lines in the OCT B-Scan (1A) indicate the inner and outer border of the retinal nerve fiber layer (RNFL) found by the algorithm. In 1C, the measured RNFL thickness is plotted on the thickness values measured in healthy subjects of the same age. The mean RNFL thickness of sectors, the peripapillaryboundle and the global mean RNFL thickness is shown in 1D.

Figure 2. Bland and Altman plot for differences in mean global RNFL thickness measured with Method A[†] (meanGA) minus Method B[*] (meanGB) in normal and glaucomatous eyes.

[*]Method B = Measurements without eye tracker and without retest software, with manual positioning of the scanning circle
[†] Method A = Measurements with eye tracker and retest software engaged

Figure 3.ΔCOVG = Difference of coefficients of variation found for both methods (Method A† minus Method B*) in measurements of the global mean RNFLthickness (G).The reduction of COV (gain of Reproducibility) is shown as a bar, together with the corresponding 95% confidence interval.

*Method B = Measurements without eye tracker and without retest software, with manual positioning of the scanning circle
† Method A = Measurements with eye tracker and retest software engaged

Figure 1.

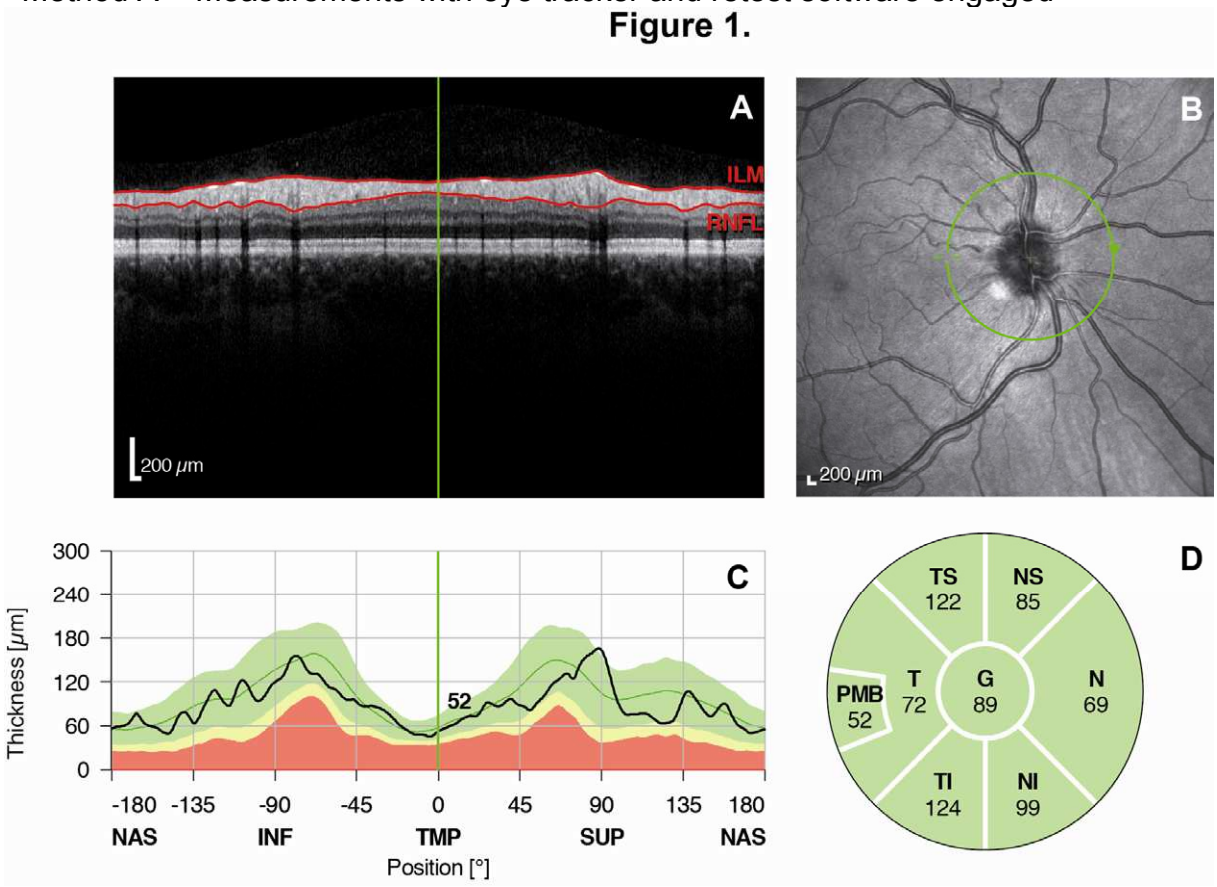

Figure 2.

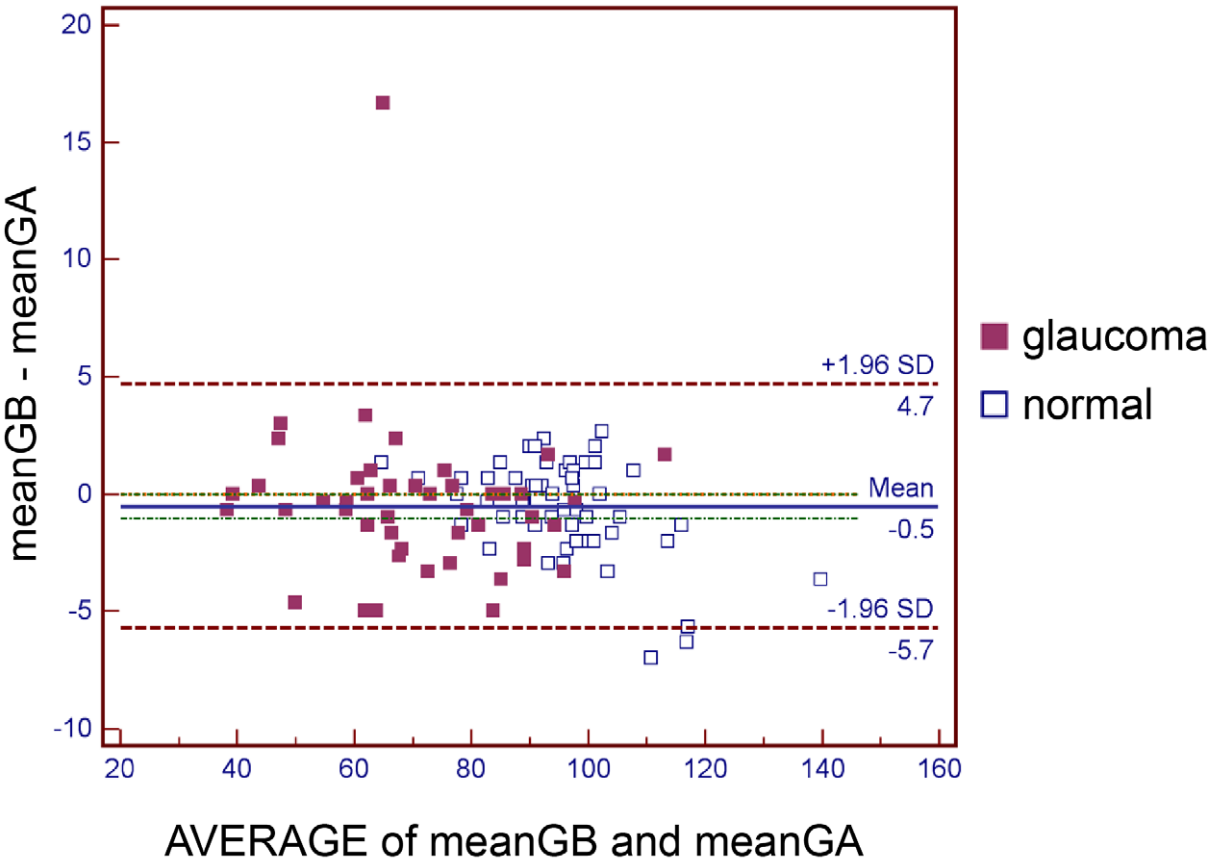

Figure 3.